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Circadian Rhythms in Locomotor Activity of the Hagfish, *Eptatretus burgeri* II. The Effect of Brain Ablation

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ABSTRACT—The hagfish, *Eptatretus burgeri*, displays locomotor activity only during the first two thirds of the dark period under 12L: 12D (7:00–19:00 light, 19:00–7:00 dark), and shows a clear free-running rhythm under constant darkness. The altered activity in the animal, whose brain was surgically removed except for the medulla oblongata, assumed a peculiar pattern which can be described as follows: (1) The free-running rhythm in constant darkness disappeared. (2) Under 12L: 12D, motor activity in the dark period disappeared, and continuous activity was observed throughout the light period. (3) This continuous activity always appeared and remained throughout the light period in various light regimens and it seems to be a direct reaction to light.

INTRODUCTION

There are many reports concerning the localization of the circadian pacemaker. It has been suggested that it is in the optic lobes of the cockroach [1] and of the cricket [2], in the prothoracic gland of the moth [3] and in the eyes of a molluscan species [4]. In vertebrates, the suprachiasmatic nucleus of the rat [5], the pineal gland of the chick [6, 7], both the suprachiasmatic nuclesus and the pineal gland of the house sparrow [8] and the pineal gland of the lamprey [9] are candidate tissues in which circadian pacemakers may be located. It is remarkable that the pineal gland is supposed to play an important role in circadian control in lower vertebrates. The hagfish, one of the most primitive vertebrates, belongs to the same vertebrate group in which the lamprey is also included. However, the hagfish is supposed not to have a pineal gland [10].

In the present study, as the first step in determining the localization of the circadian pacemaker in the animal, the effect of the brain ablation on motor activity patterns was investigated. Normally the animal shows a clear nocturnal rhythm under light-dark cycles and displays a free-running rhythm when placed in continuous darkness [11].

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MATERIALS AND METHODS

The hagfish, *Eptatretus burgeri*, were collected by use of a trap containing sardines as bait. For experiments both males and females were used, because it is not possible to distinguish males from females on the basis of outer appearance. Furthermore, males and females are similar in their circadian rhythms.

The methods and procedures for recording the motor activity of the animals were described in a previous paper [11]. The water temperature was kept at 15°C, and no food was given throughout the experiment. All surgical operations were done while the animals were lightly anesthetized with MS 222. The animal was fixed on a plastic stage, and the skin and the fibrous connective tissue covering the brain were cut longitudinally along the median axis. The brain was removed with a pair of scissors. The connective tissue was replaced as it was originally, and the skin was sewn. Ten animals were subjected to a sham-operation. In them the skin and the fibrous connective tissue were cut but the brain was not disturbed. The animals were kept in a large aquarium under 12L: 12D for two weeks prior to the recording of the behaviour in the experimental aquaria.

RESULTS

The intact animal clearly shows nocturnal swimming activity, which occurs only in the first two thirds of the dark period under 12L: 12D (7:00–19:00 light, 19:00–7:00 dark), and it displays a distinct free-running rhythm under constant darkness [11]. In the sham-operated animal activity rhythms were the same as in the intact animals both in the light-dark cycle and in constant darkness (Fig. 1).

When the entire brain was removed, the animal immediately died. However, animals, in which the medulla oblongata was left intact, survived for at least two months. They were very active, swimming in a manner similar to the intact hagfish: swimming near the surface of the water and moving along the edges of the aquarium.

The activity pattern in the brain-ablated animal was very different from that in the sham-operated one; under 12L: 12D, the activity did not occur in the dark period, but appeared continuously throughout the light period, and under constant darkness, intermittent activity with no circadian rhythm was recorded. Figure 2 showed one of the nine records from brain-ablated animals.

In Figure 3, various light-dark schedules were programmed for one brain-ablated animal. Similarly, activity was confined almost completely within light period under all the lighting conditions including 6L: 6D and 77L. No transient activity was observed when the light-dark cycle was reversed.

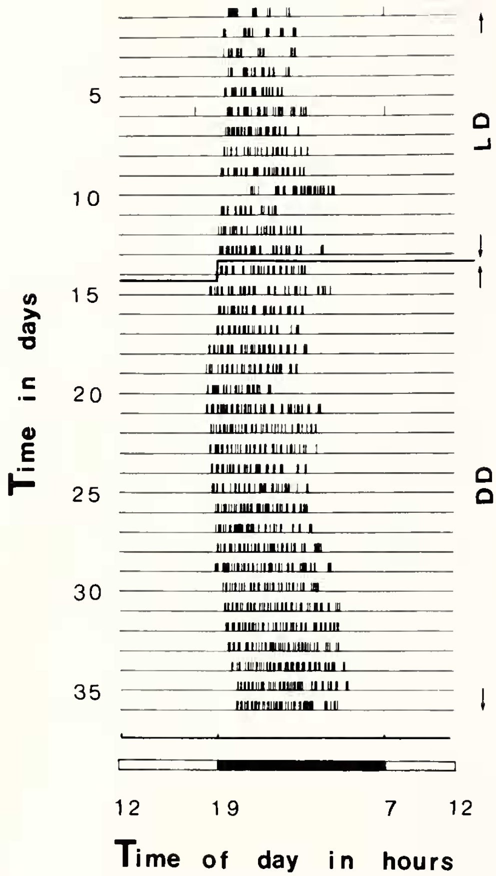


Fig. 1. Locomotor activity recorded for the sham-operated hagfish kept in 12L:12D and in constant darkness. The activity is indicated by the vertical marks on the time lines. The activity occurred only in the first two thirds of the dark period in 12L: 12D and displays a distinct free-running rhythm in constant darkness. These activity patterns are fairly the same ones as those in an intact hagfish.

DISCUSSION

Ueck and Kobayashi [12] searched unsuccessfully for a pineal gland in the hagfish, *Eptatretus burgeri*. It is of interest to find the location of the circadian pacemaker in an animal which has no pineal.

In the present study, we found that the circadian rhythm disappeared when the brain was removed, except for the medulla oblongata. This fact suggests that the circadian pacemaker may be in the brain. In these experiments locomotor activity was observed as the measurable result of the operations. Since the center controlling locomotor activity itself should exist in the brain and might be disturbed by our procedures then we cannot conclude definitively whether the circadian pace-

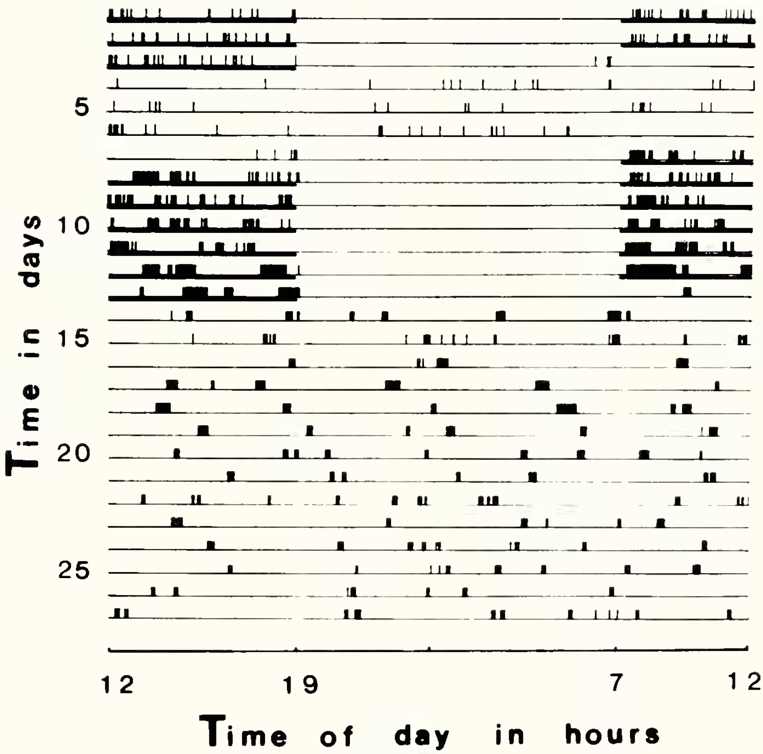
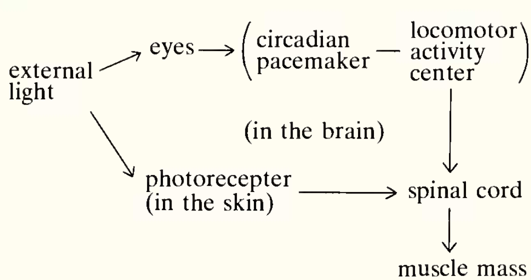


FIG. 2. Locomotor activity recorded for the operated hagfish kept in 12L: 12D and in constant darkness. The underlines show the light period. In constant darkness, the free-running rhythm disappeared and intermittent activity were recorded throughout the period. In 12L: 12D, the animal showed activity throughout the light period but none in the dark.

maker is located in the brain. Continuous swimming by animals lacking a fore- and mid-brain, however, argues that no essential motor control was impaired by the operation.

The characteristic response to light changed after removal of the fore- and mid-brain in this animal even though optic function was lost. Therefore, the locomotor activity stimulated by light stimuli probably was in response to a photoreceptor in the skin. Previous reports have established a photoreceptor in the skin of hagfish [13]. Light perceived through eyes has been supposed to control the nocturnal rhythm in intact hagfish because the nocturnal rhythm can not stay in dark period and free-runs after eye removal (unpublished data, Kabasawa and Ooka-Souda).

Accordingly, the following innervation scheme can be postulated in control of the locomotor activity system in the hagfish.



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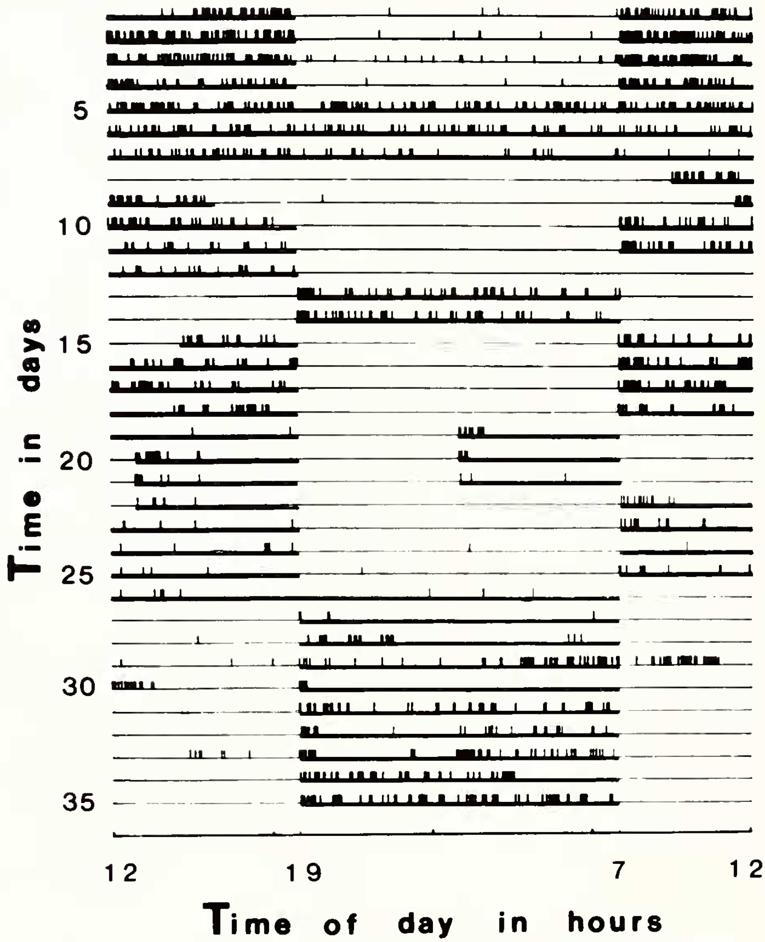

FIG. 3. Locomotor activity recorded for the operated hagfish kept under the various light-dark programs indicated. The brain-ablated animal which was under such time schedules as 12L: 12D, 77L, 21D, 12L: 12D, reversal of the 12L: 12D, 12L: 12D, 6L: 6D, 12L: 12D and reversal of the 12L: 12D successively alternatively behaved itself with light-active/dark-resting.

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